

Supporting Materials
for
QM/MM modeling of the flavin functionalization in the RutA monooxygenase

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Section S1.

Description of the files with the atomic coordinates (pdb-format) of the structures optimized in QM/MM calculations, which are deposited to the general-purpose open-access repository ZENODO (can be accessed via <https://doi.org/10.5281/zenodo.7558382>).

Complex-1.pdb - the minimum-energy structure of the triplet-state flavin-oxygen complex, which initiates pathway-1 (Fig. 2a in the manuscript).

Complex-2.pdb - the minimum-energy structure of the triplet-state flavin-oxygen complex, which initiates pathway-2 (Fig. 4a in the manuscript).

Complex-3.pdb - the minimum-energy structure of the triplet-state flavin-oxygen complex, which initiates pathway-3 (Fig. 5a in the manuscript).

Complex-4.pdb - the minimum-energy structure of the triplet-state flavin-oxygen complex, which initiates pathway-4 (Fig. 7a in the manuscript).

Complex-5.pdb - the minimum-energy structure of the triplet-state flavin-oxygen complex, which initiates pathway-5 (Fig. 9a in the manuscript).

1-FIC4a.pdb – the minimum-energy structure of the singlet-state flavin-oxygen adduct $\text{Fl}_{\text{C}4\text{aOO}}^-$ located on the pathway-1 (Fig. 2b in the manuscript).

2-Flox.pdb – the singlet-state minimum-energy structure of the oxidized flavin with the nearby protonated superoxide located on the pathway-2 (Fig. 4b in the manuscript).

3-Flox.pdb – the singlet-state minimum-energy structure of the oxidized flavin with the nearby hydrogen peroxide and hydroxyl located on the pathway-3 (Fig. 5c in the manuscript).

4-FlN5O.pdb – the minimum-energy structure of the singlet-state flavin-oxygen adduct Fl_{N5O} located on the pathway-4 (Fig. 8a in the manuscript).

4-FlN5O-C8.pdb – the minimum-energy structure of the singlet-state flavin-oxygen adduct Fl_{N5O} hydrated at the C8 position, which is located on the pathway-4 (Fig. 8b in the manuscript).

5-FlC6.pdb – the minimum-energy structure of the singlet-state flavin-oxygen adduct Fl_{C6OOH} located on the pathway-5 (Fig. 9b in the manuscript).

5-FlC6C7.pdb – the minimum-energy structure of the singlet-state flavin-oxygen adduct with the C6-C7-epoxide located on the pathway-5 (Fig. 9c in the manuscript).

Section S2.

A sample input file for the QM/MM optimization using the NWChem program

```
title "f14sb"
start f14sb
echo

charge -4
memory total 3000 mb
#print debug
SCRATCH_DIR /tmp
PERMANENT_DIR .

prepare
    directory_3 ../
    system f14sb
    source ./f14sb-F.pdb
    amber
    new_top new_seq
    new_rst
    modify atom 1:_N1 quantum
    modify atom 1:_H1 quantum
    modify atom 1:_C2 quantum
    modify atom 1:_O2 quantum
    modify atom 1:_N3 quantum
    modify atom 1:_H3 quantum
    modify atom 1:_C4 quantum
    modify atom 1:_O4 quantum
    modify atom 1:_C4A quantum
    modify atom 1:_N5 quantum
    modify atom 1:_C5A quantum
    modify atom 1:_C6 quantum
    modify atom 1:_H6 quantum
    modify atom 1:_C7 quantum
    modify atom 1:_C7M quantum
    modify atom 1:_H71 quantum
    modify atom 1:_H72 quantum
    modify atom 1:_H73 quantum
    modify atom 1:_C8 quantum
    modify atom 1:_C8M quantum
    modify atom 1:_H81 quantum
```

modify atom	1:_H82	quantum
modify atom	1:_H83	quantum
modify atom	1:_C9	quantum
modify atom	1:_H9	quantum
modify atom	1:_C9A	quantum
modify atom	1:_N10	quantum
modify atom	1:_C10	quantum
modify atom	1:_C1*	quantum
modify atom	1:2H1*	quantum
modify atom	1:3H1*	quantum
modify atom	1:_C2*	quantum
modify atom	1:_H2*	quantum
modify atom	1:_O2*	quantum
modify atom	1:2HO*	quantum
modify atom	1:_C3*	quantum
modify atom	1:_H3*	quantum
modify atom	1:_O3*	quantum
modify atom	1:3HO*	quantum
modify atom	1:_C4*	quantum
modify atom	1:_H4*	quantum
modify atom	1:_O4*	quantum
modify atom	1:4HO*	quantum
modify atom	1:_C5*	quantum
modify atom	1:2H5*	quantum
modify atom	1:3H5*	quantum
modify atom	1:_O5*	quantum
modify atom	1:_P	quantum
modify atom	1:_O1P	quantum
modify atom	1:_O2P	quantum
modify atom	1:_O3P	quantum
modify atom	105:_CB	quantum
modify atom	105:_HB	quantum
modify atom	105:_CG2	quantum
modify atom	105:2HG2	quantum
modify atom	105:3HG2	quantum
modify atom	105:4HG2	quantum
modify atom	105:_OG1	quantum
modify atom	105:_HG1	quantum
modify atom	134:_CB	quantum
modify atom	134:2HB	quantum
modify atom	134:3HB	quantum
modify atom	134:_CG	quantum
modify atom	134:_OD1	quantum
modify atom	134:_ND2	quantum
modify atom	134:2HD2	quantum
modify atom	134:3HD2	quantum
modify atom	139:_CB	quantum
modify atom	139:2HB	quantum
modify atom	139:3HB	quantum
modify atom	139:_CG	quantum
modify atom	139:_CD1	quantum
modify atom	139:_HD1	quantum
modify atom	139:_NE1	quantum
modify atom	139:_HE1	quantum
modify atom	139:_CE2	quantum
modify atom	139:_CZ2	quantum
modify atom	139:_HZ2	quantum
modify atom	139:_CH2	quantum
modify atom	139:_HH2	quantum
modify atom	139:_CZ3	quantum

modify atom	139:_HZ3	quantum
modify atom	139:_CE3	quantum
modify atom	139:_HE3	quantum
modify atom	139:_CD2	quantum
modify atom	292:_CG	quantum
modify atom	292:2HG	quantum
modify atom	292:3HG	quantum
modify atom	292:_CD	quantum
modify atom	292:_OE1	quantum
modify atom	292:_OE2	quantum
modify atom	400:_OX1	quantum
modify atom	400:_OX2	quantum
modify atom	501:2HW	quantum
modify atom	501:_OW	quantum
modify atom	501:3HW	quantum
modify atom	502:2HW	quantum
modify atom	502:_OW	quantum
modify atom	502:3HW	quantum
modify atom	503:2HW	quantum
modify atom	503:_OW	quantum
modify atom	503:3HW	quantum
modify atom	504:2HW	quantum
modify atom	504:_OW	quantum
modify atom	504:3HW	quantum
modify atom	505:2HW	quantum
modify atom	505:_OW	quantum
modify atom	505:3HW	quantum
modify atom	506:2HW	quantum
modify atom	506:_OW	quantum
modify atom	506:3HW	quantum
modify atom	507:2HW	quantum
modify atom	507:_OW	quantum
modify atom	507:3HW	quantum
modify atom	508:2HW	quantum
modify atom	508:_OW	quantum
modify atom	508:3HW	quantum
modify atom	509:2HW	quantum
modify atom	509:_OW	quantum
modify atom	509:3HW	quantum
modify atom	510:2HW	quantum
modify atom	510:_OW	quantum
modify atom	510:3HW	quantum
modify atom	511:2HW	quantum
modify atom	511:_OW	quantum
modify atom	511:3HW	quantum
modify atom	512:2HW	quantum
modify atom	512:_OW	quantum
modify atom	512:3HW	quantum
modify atom	513:2HW	quantum
modify atom	513:_OW	quantum
modify atom	513:3HW	quantum
modify atom	628:2HW	quantum
modify atom	628:_OW	quantum
modify atom	628:3HW	quantum
modify atom	2811:2HW	quantum
modify atom	2811:_OW	quantum
modify atom	2811:3HW	quantum
modify atom	514:2HW	quantum
modify atom	514:_OW	quantum
modify atom	514:3HW	quantum

```

modify atom 600:2HW quantum
modify atom 600:_OW quantum
modify atom 600:3HW quantum
modify atom 619:2HW quantum
modify atom 619:_OW quantum
modify atom 619:3HW quantum
modify atom 2824:2HW quantum
modify atom 2824:_OW quantum
modify atom 2824:3HW quantum
update lists
ignore
write fl4sb.rst
write fl4sb.pdb
end

task prepare

driver
    tight
end

md
    system fl4sb
    noshake solute
    cutoff 10 qmmm 10
    msa 30000
end

basis
#      * library "cc-pvdz"
      * library "6-31G*"
end

dft
    xc pbe0
    disp vdw 3
    iterations 250
    mult 1
    grid
end

driver
    trust 0.05
    tight
end

qmmm
    region qmlink mm_solute
    method bfgs lbfqs
    maxiter 5 100
    density espfit
    ncycles 100
    convergence 1.0d-7
    bqzone 50
end

constraints
#    spring bond 100 101 1.0 3.5
end

```

```

task qmmm dft optimize

prepare
    system fl4sb
    read rst fl4sb.rst
    write solute pdb fl4sb-F.pdb
end

task prepare

```

Section S3.

A sample input file for CASSCF and XMCQDPT2 calculations using the Firefly program.

```

!
$intgrl extfi=.t. $end
$MCQGENS GEN1=3 GEN2=3 GEN3=1 USEGEN1=.T. $END
$xmcqdpt halloc=.t. ri=.t. alttrf(1)=1,1,1,1 bigcas=1 $end
$rimp2 auxbas=def2-TZVPPD/C extfil=.t. $end
$gugem pack2=1 $end
$p2p p2p=1 dlb=1 $end
$smp smppar=1 np=10 mklnp=10 $end
$trans dirtrf=.t. aoints=dist altpar=.t. mptran=2 mode=112 $end
!
$CONTRL SCFTYP=mcsfc RUNTYP=energy INTTYP=HONDO ICUT=10
    icharg=-1 mult=1
    maxit=350
!    dfotyp=pbe0
    mplevel=2
!    nzvar=1
$END
$ZMAT DLC=.T. AUTO=.T.
    NONVDW(1)= 10,48, 48,60, 48,77, 77,68, 77,75,
                75,51, 51,42, 42,45, 45,62, 62,65,
                56,62, 34,53, 34,72
!        ifzmat(1)=1,7,77
$END
$system TIMLIM=90000 mwords=300 $end
!
! Note, high computation accuracy is enforced throughout
!
$trans dirtrf=.t. mptran=2 mode=0 cutrf=1.0d-10 $end
$statpt nstep=1000 opttol=1.d-4 projct=.f.
!    dxmax=0.02 trmax=0.02
!    ifreez(1)=1,2,3, 118,119,120, 130,131,132,
!                262,263,264, 178,179,180, 235,236,237,55, 56,57
$END
```

```

$SCF DIIS=.t. SOSCF=.f. DIRSCF=.T. $END
$BASIS gbasis=cc-pvdz extfil=.t. $END
! $BASIS GBASIS=N31 NGAUSS=6 ndfunc=1 $END
! $dft nrad0=96 nleb0=302 $end
$DRT GROUP=C1 Nmcc=170 NDOC=7 NALP=0 NVAL=3 FORS=.T. $END
$GUGDIA NSTATE=20 ITERMX=3200 $END
$gugdm2 wstate(1)=1,1,1,1,1, 1,1,1,1,1, 1,1,1 $end
$MCSCF cistep=guga
!   istate=2 acurcy=1.0d-7 npflg(9)=1
      SOSCF=.T. FULLNR=.F. FCORE=.F. maxit=20
$END
$xmcqdpt istsym=1 kstate(1)=1,1,1,1,1, 1,1,1,1,1, 1,1,1,1,1
  irot=2 edshft=0.02 avecoe(1)=1,1,1,1,1, 1,1,1,1,1, 1,1,1,1,1
  inorb=0
$end
$GUESS GUESS=MOREAD NORB=180 norder=0
  norder(173)=170    norder(170)=173
$END
$NBO reson aonbo=w $END
$license nbolid=1 nbokey=57c9e756 $end
$DATA

```

C1

N	7.0	96.174	77.625	-3.754
H	1.0	92.342	80.461	-2.037
C	6.0	95.628	77.798	-4.973
O	8.0	96.262	77.507	-6.004
N	7.0	94.333	78.282	-5.105
H	1.0	93.882	78.117	-6.008
C	6.0	93.589	78.826	-4.089
O	8.0	92.463	79.270	-4.239
C	6.0	94.297	78.820	-2.786
N	7.0	93.733	79.406	-1.765
C	6.0	94.270	79.251	-0.536
C	6.0	93.585	79.752	0.585
H	1.0	92.670	80.316	0.437
C	6.0	94.013	79.482	1.864
C	6.0	93.120	79.869	3.006
H	1.0	93.607	79.779	3.978
H	1.0	92.247	79.206	3.016
H	1.0	92.725	80.880	2.873
C	6.0	95.254	78.802	2.044
C	6.0	95.822	78.585	3.415
H	1.0	96.818	78.141	3.362
H	1.0	95.180	77.916	4.000
H	1.0	95.898	79.530	3.964
C	6.0	95.972	78.349	0.948

H	1.0	96.936	77.884	1.120
C	6.0	95.465	78.504	-0.348
N	7.0	96.076	77.974	-1.462
C	6.0	95.535	78.108	-2.697
C	6.0	97.392	77.366	-1.328
H	1.0	97.389	76.706	-0.460
H	1.0	97.572	76.757	-2.208
C	6.0	98.433	78.480	-1.231
H	1.0	98.228	79.085	-0.345
O	8.0	98.311	79.359	-2.329
H	1.0	98.725	78.946	-3.125
C	6.0	99.835	77.914	-1.082
H	1.0	100.522	78.764	-1.140
O	8.0	100.211	77.068	-2.157
H	1.0	99.788	76.186	-2.051
HL	1.0	99.986	77.410	-0.127
O	8.0	91.491	80.953	-1.904
O	8.0	90.719	80.031	-1.129
H	1.0	93.955	76.439	-8.369
O	8.0	93.780	77.277	-7.884
H	1.0	92.826	77.466	-8.006
H	1.0	98.382	74.329	-1.204
O	8.0	99.234	74.520	-1.676
H	1.0	99.157	74.042	-2.515
H	1.0	91.174	76.774	-0.885
O	8.0	90.538	75.366	-0.382
H	1.0	91.088	75.005	0.353
H	1.0	91.844	76.767	-4.870
O	8.0	91.772	76.703	-5.838
H	1.0	92.425	76.005	-6.021
H	1.0	93.875	72.083	-3.383
O	8.0	93.241	72.536	-4.000
H	1.0	92.578	73.035	-3.441
H	1.0	93.394	75.735	-4.085
O	8.0	92.923	76.086	-3.295
H	1.0	92.263	75.291	-3.060
H	1.0	94.033	74.432	-1.108
O	8.0	94.590	75.239	-1.331
H	1.0	94.103	75.634	-2.097
H	1.0	94.024	76.059	0.146
O	8.0	93.466	76.474	0.843
H	1.0	92.838	76.989	0.298
H	1.0	96.438	73.574	0.133
O	8.0	96.868	74.145	-0.537
H	1.0	96.114	74.592	-0.991
H	1.0	93.457	72.384	-0.800

O 8.0 92.997 73.270 -0.705
H 1.0 92.317 73.404 -1.435
H 1.0 93.727 73.823 -4.861
O 8.0 93.741 74.617 -5.476
H 1.0 94.644 74.673 -5.869
H 1.0 92.490 73.762 0.798
O 8.0 92.153 74.254 1.592
H 1.0 92.670 75.098 1.530
H 1.0 92.186 77.220 -1.997
O 8.0 91.768 77.534 -1.172
H 1.0 91.130 79.132 -1.284
H 1.0 90.806 74.833 -1.172
O 8.0 91.577 74.117 -2.622
H 1.0 90.696 73.923 -2.970
H 1.0 94.955 78.635 -8.245
O 8.0 95.812 79.114 -8.237
H 1.0 96.230 78.675 -7.475
H 1.0 95.932 75.999 -6.713
O 8.0 95.911 75.051 -6.971
H 1.0 95.275 74.985 -7.747
H 1.0 90.789 81.054 0.429
O 8.0 91.142 81.711 1.057
H 1.0 91.503 82.396 0.476
\$END